PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PBA/P089368PWO	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/001803	International filing date *(day/month/year)* 23.04.2004	Priority date *(day/month/year)* 25.04.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant THE UNIVERSITY OF MANCHESTER et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 1 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.02.2005	05.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hennard, C Telephone No. +49 89 2399-7355

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/001803

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-49	as originally filed

Claims, Numbers

7 (part), 8-46	as originally filed
1-6, 7 (part)	received on 25.02.2005 with letter of 25.02.2005

Drawings, Sheets

1/44-44/44	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-46
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-46
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-46
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:
   D1: US-B-6 475 730

2. **Novelty (Article 33(2) PCT):**
   No document of the cited prior art discloses the use of 2,2,2-trifluoroethanol, ethylene glycol or ethylene glycol dimethyl ether for enhancing exciplex formation in a nucleic acid hybridisation assay or a method for detecting an exciplex involving one of these solvents. Therefore, **claims 1-46** of the present application are considered novel and fulfil the requirements of **Article 33(2) PCT**.

3. **Inventive merit (Article 33(3) PCT):**
   Considering the argumentation provided with the letter of 25.02.2005, the following opinion is given:
   D1 (claims), which is the closest prior art, concerns the detection of the presence of a polynucleotide in a sample involving the detection of exciplexes. The use of present **claim 1** distinguishes itself from **D1** by the presence in the sample during the exciplex measurement of a solvent selected from 2,2,2-trifluoroethanol, ethylene glycol or ethylene glycol dimethyl ether.
   The technical effect achieved by the addition of the the solvent, as illustrated by the comparative tests provided, is an increase in the exciplex signal during the hybridisation assay. Thus, the problem to be solved by the present claim 1 can be seen in the provision of a method for increasing the detection signal of an exciplex in a nucleic acid hybridisation assay.
   Since no cited prior art describes the increase of exciplex signal when a solvent selected from 2,2,2-trifluoroethanol, ethylene glycol or ethylene glycol dimethyl ether is added in the nucleic acid hybridisation assay conditions, an inventive merit can be recognised in the use as characterised in **claim 1** because the skilled person would find no incentive in the prior art to add such a solvent in order to increase the exciplex signal.
   The same reasonning applies to the independent **claims 3 and 7** which thus demonstrate an inventive merit.
   It is therefore concluded that **claims 1-46** of the present application involve an inventive

merit and fulfil the requirements of **Article 33(3) PCT.**

4. **Industrial applicability (Article 33(4) PCT):**
An industrial applicability of the invention is obvious and **claims 1-46** of the present application are considered to fulfil the requirements of **Article 33(4) PCT.**

CLAIMS

1. The use of an organic solvent selected from 2,2,2-trifluoroethanol, ethylene glycol or ethylene glycol dimethyl ether for enhancing formation, potential formation, fluorescence and/or detection of an exciplex in a nucleic acid hybridisation assay.

2. The use as claimed in claim 1 wherein the solvent is 2,2,2-trifluoroethanol.

3. A method of analysis which is a nucleic acid hybridisation assay involving detection of an exciplex in a medium containing exciplex forming partners, the method comprising photoirradiating the medium at the appropriate wavelength and detecting for formation of an exciplex characterised in that on photoirradiation the medium contains an organic solvent selected from 2,2,2-trifluoroethanol, ethylene glycol or ethylene glycol dimethyl ether.

4. A method as claimed in claim 3 wherein the medium is a liquid medium and on photoirradiation contains more that 30%, e.g. more than 50%, by volume of said solvent.

5. A method as claimed in claim 4 wherein the liquid medium contains 60% to 99% by volume of the solvent.

6. A method as claimed in any one of claims 3 to 5 wherein the solvent is 2,2,2-trifluoroethanol.

7. A method of analysing a sample to determine the presence or otherwise therein of a target polynucleotide sequence, the method comprising

(a) treating the sample under hybridising conditions with